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COMMENT

# Comment on Grahl-Nielsen et al. (2003) 'Fatty acid composition of the adipose tissue of polar bears and of their prey: ringed seals, bearded seals and harp seals'

**Gregory W. Thiemann[1,*], Suzanne M. Budge[2], W. Don Bowen[3], Sara J. Iverson[1]**

[1]Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, Nova Scotia B3H 4J1, Canada
[2]Department of Food Science and Technology, Dalhousie University, 1360 Barrington Street, Halifax, Nova Scotia B3J 2X4, Canada
[3]Marine Fish Division, Bedford Institute of Oceanography, 1 Challenger Drive, Dartmouth, Nova Scotia B2Y 4A2, Canada

Accurately determining the diet of free-ranging carnivores poses significant challenges. Limitations of both fecal and stomach content analyses are well-known and have led to the development of methods which attempt to overcome some of these problems. One such method is the use of predator fatty acid (FA) profiles (e.g. Iverson 1988). Fatty acids contain information that can be used both qualitatively, to infer changes in diet without specifying the types of prey consumed (e.g. Iverson et al. 1997a), and more recently, quantitatively, where the proportion of prey types consumed is estimated using FA data from the predator and potential prey and an understanding of the effects of predator lipid metabolism (Iverson et al. 2004).

In a recent paper, Grahl-Nielsen et al. (2003) propose investigating the FA composition of polar bears *Ursus maritimus* and of several of their seal prey with a number of objectives, one of which is to 'explore the possibility of using FAs to determine the diet of polar bears' (p. 276). The authors find (p. 275) that polar bear FA profiles differ from those of any of the seal species and therefore conclude that 'polar bear adipose tissue has a unique FA composition' and that 'selective processes' within polar bears modify ingested FAs to such an extent that FA analysis can provide no useful information about diet. Grahl-Nielsen and colleagues have come to similar conclusions about the value of FAs to investigate the diets of other predators (Grahl-Nielsen & Mjaavatten 1991, Grahl-Nielsen et al. 2000, Olsen & Grahl-Nielsen 2003). We contend that these conclusions are incorrect and are based partly on inappropriate sampling of tissue FAs and invalid statistical analyses (see also Smith et al. 1999), but mainly on a misunderstanding of vertebrate FA metabolism and of the principles underlying the use of FAs to investigate predator diets.

**Sampling of tissue FAs and statistical analyses.** One of the most alarming aspects of Grahl-Nielsen et al.'s (2003) study is the gross inflation of the sample sizes of polar bear and seal fat samples used in the analyses (Table 1). The lipid tissue collected from each animal was subsampled at multiple depths to examine variability within individuals, but all subsamples were treated as independent samples in all analyses (e.g. Fig. 1 and Table 1 in Grahl-Nielsen et al. 2003). In the most flagrant case, only 10 harp seals *Phoca groenlandica* were used to generate the 195 samples reported. Clearly, those 195 samples, and the inflated number of samples from the other prey species and polar bears (Table 1), are not statistically independent and cannot be used to estimate an appropriate standard error on which to base inferential conclusions. The result of this sample inflation is to seriously underestimate the standard errors and thus overestimate the statistical significance of tested differences. With no indication of the actual variability present among the sampled animals, we contend that the data presented have little value beyond providing a rough estimate of the mean percentage of each FA.

Table 1. Inflation of sample sizes for polar bears and seal prey used for data presentation and statistical analyses in Grahl-Nielsen et al. (2003)

| | No. of individuals | No. of samples ind.$^{-1}$ | Total no. reported |
|---|---|---|---|
| Polar bear | 18 | 3 | 54 |
| Harp seal | 10 | ~19 | 195 |
| Ringed seal | 10 | ~9 | 90 |
| Bearded seal | 9 | ~14 | 125 |

*Email: thiemann@dal.ca

Another problem with the study of Grahl-Nielsen et al. (2003) lies in the non-representative sampling of FAs from the predator and prey lipid tissues (see Thiemann et al. 2004). From each polar bear fat biopsy, the authors take 3 small subsamples from different depths along the core in an attempt to examine FA variability within individuals. Although the subsamples taken in this study (10 to 20 mg) are larger than the 2 mg subsamples used in previous studies (e.g. Grahl-Nielsen & Mjaavatten 1991, Grahl-Nielsen et al. 2000), there is still no reason to expect that such small samples can be taken in a way to provide a representative estimate of the FA composition of the entire sample collected. A more appropriate test of the difference between inner, middle, and outer tissue lipids in polar bears would be to divide each biopsy into 3 equal subsamples, extract all FAs from each of these, and then quantify the representative FA profiles of each section.

Certainly, there is value in repeatedly sampling the same individual to test hypotheses concerning FA composition as a function of tissue depth or body location, but the approach used in this study is invalid and therefore conclusions about how FAs may vary by depth in adipose tissue are also unwarranted. Profile analysis or mixed-effects, repeated-measure models would properly account for the correlation among samples and allow more confident conclusions; however, we note that considerably larger numbers of individuals would be needed to effectively use these methods than were obtained for this study.

Elsewhere (Grahl-Nielsen & Mjaavatten 1991, Grahl-Nielsen 1999) and in the paper under discussion, the authors stress the importance of a multivariate approach to FA analyses. Therefore, it is disappointing that Grahl-Nielsen et al. (2003) rely almost exclusively on multiple univariate comparisons to make their case that polar bear and seal FA profiles differ. The authors state that '[f]ifteen of the 28 FAs analyzed were found in lower relative amounts in the polar bears than in any of the 3 seal species' (p. 275). They also found that '[t]he FA composition of the blubber of each seal species was significantly different from that of the inner adipose tissue of the polar bears ($p < 0.01$) for all 28 FAs' (p. 278). Although one would expect polar bear adipose tissue FA composition to have significant differences in levels of specific FAs from that of their prey (see below), the reader is given no information about which tests were used to support these statements.

In their examination of potential stratification in polar bear FA stores, the authors appear to have (i.e. no information is provided in the paper) again inappropriately used multiple univariate comparisons without any table-wide control for alpha (see Rice 1989). The single multivariate analysis (PCA) done on these stratification data was 'carried out on the basis of the 10 FAs that were significantly different between layers (Table 1)' (p. 278) based on previous multiple univariate tests. This analysis concluded that those differences became more prominent. This is hardly surprising. Unfortunately, it is also an invalid approach to the analysis of data.

We also point out, as before (see Smith et al. 1999), that Grahl-Nielsen and colleagues are using PCA in a rather odd and uninformative way. PCA is a multivariate technique that is often used to reduce the dimensions of multivariate data via linear combinations of variables. The first 2 or 3 of these combinations are then used to graphically display the relationships among individuals based on these correlated sets of the original variables, or they can be used in further statistical analyses. In order to do this, the covariance or correlation matrix of the observations is used. Application of PCA to the covariance matrix assumes that we have a statistically valid estimate of that matrix, which is only possible if $n \geq p$, where $n$ is the number of independent samples and $p$ is the number of variables. Grahl-Nielsen et al. (2003) achieve this by using the inflated, non-independent subsamples referred to above. Although one might argue that for exploratory analysis this violation can be tolerated, Grahl-Nielsen et al. (2003) are not using PCA in this way and therefore their inferences are invalid.

**Details of FA metabolism and use of FAs to investigate predator diets.** Although aspects of the paper pertaining to tissue sampling and statistical analysis are troubling and cast serious doubt on the authors' conclusions, the far greater problem is the apparent misunderstanding of the expected relationship between predator and prey FA compositions. While the quantitative application of FAs to diet estimation is relatively new (Iverson et al. 2004), it has long been known that FA patterns are modified in certain ways in all monogastric vertebrate endotherms, and in ways that are similar and predictable among species (e.g. Nelson 1992). Polar bears are no exception.

As noted previously, FAs can be used in 2 ways to study foraging ecology and diets:

(1) When used qualitatively, only the FA composition of the predator need be considered. Here one is simply asking if there are temporal or spatial differences in diet without attempting to specify which prey species are consumed. This technique is based on the knowledge that FA structures are transferred largely unaltered across trophic levels—a fact that has been established through decades of biochemical and ecological research (e.g. Klem 1935, Ackman & Eaton 1966, Hooper et al. 1973, Ackman 1980, Iverson 1993, Kirsch et al. 1998, Lea et al. 2002, Bradshaw et al. 2003). The FA composition of the diet has been shown to influence predator FAs in a wide variety of consumers, in-

cluding zooplankton (e.g. Graeve et al. 1994, 2001), benthic invertebrates (e.g. Kharlamenko et al. 1995, Caers et al. 1999), marine and freshwater fishes (e.g. Ackman et al. 1975, Schwalme 1992, Kirsch et al. 1998), jellyfish (Fukuda & Naganuma 2001), marine and freshwater turtles (Ackman et al. 1971, Holland et al. 1990), Arctic foxes (Pond et al. 1995), black bears (Iverson et al. 2001), seabirds (e.g. Raclot et al. 1998, Iverson & Springer 2002), baleen whales (Ackman & Eaton 1966), several species of pinnipeds (e.g. Iverson 1988, 1993, Smith et al. 1996, Iverson et al. 1997a,b, Bradshaw et al. 2003), and polar bears (Colby et al. 1993). Nevertheless, most if not all of these studies also acknowledge that predator FA profiles will be influenced by biosynthesis of certain FAs and by reduced deposition of other FAs. The fact that polar bear adipose tissue FA composition does not match that of their prey is indeed quite expected. FA patterns in adipose tissue of polar bears feeding on different prey differ dramatically and if used appropriately (that is, acknowledging expected FA metabolism), these patterns can be used to estimate polar bear diets quite well (Iverson et al. in press).

(2) To use FAs quantitatively, both predator and prey FAs are required. Grahl-Nielsen et al. (2003) attempt to tackle this more ambitious task, but their attempt is seriously flawed. Iverson et al. (2004) list 4 requirements which must be fulfilled in order to estimate the diet of a predator based on FA profiles: (1) an understanding of, and correction for, the effects of predator lipid metabolism on FA deposition, (2) consideration of variability in FA composition within and among prey species, (3) appropriate sampling and chemical analysis of predator and prey lipid tissue, and (4) a statistical estimation model (quantitative fatty acid signature analysis, QFASA). The study by Grahl-Nielsen et al. (2003) satisfies none of these requirements.

As stated above, the FA composition of the predator is not expected to match that of its prey. First, the predator will invariably synthesize certain specific FAs endogenously. These FAs will appear in larger amounts in the predator than in the diet and will serve to reduce the relative levels of others, because FA concentrations are commonly expressed as percentages. Further, certain other ingested FAs will be preferentially metabolized prior to deposition and therefore will appear in absolutely smaller amounts in the predator than in the diet (Cooper et al. 2003, Iverson et al. 2004). Controlled studies conducted on several species of phocid seals, otariids seals, seabirds and captive mink *Mustela vison* have shown that patterns are similar and predictable among monogastric vertebrate endotherms (e.g. Kirsch et al. 2000, Iverson & Springer 2002, Cooper et al. 2003, Iverson et al. 2004, in press, unpubl., Tollit et al. 2003).

The important point here is that individual FAs are deposited from diet in a predictable way that is consistent among most predators. Iverson et al. (2004) have developed 'calibration coefficients' to account for FA metabolism within the predator. These calibration coefficients weight specific FAs according to whether they are preferentially utilized or endogenously synthesized within the predator. Iverson et al. (2004) show that when predator metabolism is considered, QFASA can provide good estimates of predator diets. Conversely, when metabolic processes are ignored, inaccurate estimates are produced. Unfortunately, Grahl-Nielsen et al. (2003) have made precisely this error. Using unidentified statistical tests, they found that polar bears differed from the 3 seal species in every one of the 28 FAs they examined. However, this does not adequately support the authors' conclusion that predator FA stores are independent of diet. They have simply neglected to account for the effect of predator metabolism on FA composition. In fact, their conclusion is puzzling considering the authors' own contention that '[c]hanges in the seals' diet may also have an impact on the composition of the blubber' (p. 280). Why diet should affect the adipose tissue stores of seals, but not of polar bears, is not explained, nor is it possible to conceive an explanation. The authors' contention that 'seal blubber provides the bears with a dietary mixture of FAs that is too fluid to be used directly as building blocks for the triacylglycerols in bears' (p. 281) is an implausible statement with no empirical or theoretical basis.

QFASA is one quantitative approach to estimating predator diets using FAs; there may be others. Yet, it is likely that any such approach must aim to estimate the diet by selecting the proportion of prey species which best fits the observed proportion of FAs in the predator tissue after appropriate consideration of FA metabolism. Given the number of FAs, variability in FA composition within and among prey, and the effects of predator metabolism on FA deposition, diet estimation can only be done using a quantitative model. Thus, it is inappropriate to suggest that simple comparisons (by eye or otherwise), such as those presented by Grahl-Nielsen et al. (2003), between predator and prey FA compositions can tell us anything about the extent to which FAs can be used to determine predator diets.

In conclusion, Grahl-Nielsen et al. (2003) fail to properly 'explore the possibility of using FAs to determine the diet of polar bears' (p. 276) because they: (1) fail to account for the expected metabolism of FAs by mammalian predators (in this case polar bears), (2) use inappropriate tissue sampling protocols, (3) carry out invalid statistical analyses, and (4) fail to use an appropriate statistical estimation model. These 4 aspects represent the minimum requirements for the quantitative estimation of any predator diet using FAs.

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